Spontaneous and testosterone-induced motility of isolated guinea-pig epididymis

Spontaneous motility[a]			Testosterone-induced motility[a, b]					
ICT[c] (mg)	RC[d] (C/10 min)	TTC[e] (mg)	ICT[f] (mg)		RC[d] (C/10 min)		TTC[e] (mg)	
			8.5 m*M*	17.0 m*M*	8.5 m*M*	17.0 m*M*	8.5 m*M*	17.0 m*M*
59.0 ± 8.8	13.5 ± 1.5	0	41.0 ± 9.7	56.0 ± 17.3	11.0 ± 1.4	17.0 ± 4.3	205.0 ± 32.0	290 ± 33.0
(37)	(37)	(37)	(10)	(6)	(10)	(6)	(10)	(6)

[a] Mean values ± SEM; figures in the parentheses indicate the number of isolated epididymis studied. [b] Final concentrations in the tissue bath solution. [c] Isometric contractile tension (ICT) of phasic cycles of tension development followed by a complete relaxation down to the point of the tissue resting or basal tension. [d] Rate of contraction (RC) represents the number of phasic contractions (C) in a period of 10 min. [e] Tension of tonic contracture (TTC) developed above the resting or basal tension of the tissue, i.e. the mg developed in a tonic sustained contraction. [f] Testosterone-induced, isometric contractile tension (ICT) of superimposed phasic cycles i.e. a process of tension development followed by a complete relaxation down to the point of TTC elicited by testosterone which is always above the resting or basal tension of tissue.

(Table). The tonic contractile activity elicited by testosterone appeared in most cases after few minutes following its addition. b) Effect of indomethacin, phentolamine or atropine on the contractile response induced by testosterone in cauda epididymis. The repeated addition of testosterone, up to 3 consecutive times, each one preceeded by several washings, had a stimulating influence comparable to that already described in the previous section. Figure 2 shows that this effect of testosterone was blocked by phentolamine ($n = 10$) or indomethacin ($n = 9$), but not by atropine ($n = 6$). On the contrary, neither phentolamine nor indomethacin altered the stimulating influence of $PGF_2\alpha$ on epididymal motility.

Discussion. In the present study the in vitro existence of spontaneous motility in cauda epididymis isolated from guinea pig was demonstrated for the first time. The contractile activity had phasic characteristics, and no spontaneous increments of basal tonic tension were observed.

Numerous papers documented that testosterone has an inhibitory effect in vivo and in vitro over the functioning of the smooth muscle of some organs of the male reproductive tract, such as seminal vesicles, vas deferens and testes[6, 10–14]. On the contrary, in the rat it has been found that testosterone seems to be important for the maintenance of epididymal motility[4–15]. We have observed that this hormone produced a stimulation of epididymal motility characterized by a distinct increase of its tonic tension. The superimposed testosterone-induced ICT was similar in magnitude and frequency to that of the spontaneous epididymal contractions. On the other hand, the stimulating effect of testosterone documented in the present study seems to be indirect; indeed it was abolished by the presence of indomethacin, a well-known inhibitor of the synthesis of prostaglandins[16] and by phentolamine, an alpha adrenergic receptor blocking agent[17]. It must be stressed that the inhibitory influence of these agents upon the effects of testosterone is not non-specific, because both drugs failed to alter epididymal contractile responses to PGF_2-α.

The present experimental results suggest that testosterone stimulates cauda epididymal smooth muscle by means of an indirect mechanism, presumably associated with endogenous noradrenaline and/or prostaglandins. Furthermore, the participation of cholinergic influences does not seem to play any role in the inotropic effect of testosterone[18].

Résumé. Les auteurs décrivent la mobilité spontanée de la partie caudale isolée de l'épididyme du cobaye. Cette mobilité se caractérise par des contractions cycliques régulières et fasciculées. Le testostérone déclanche une contraction soutenue avec des phases cycliques surajoutées, bloquées par la phentolamine ou l'indométhacine, mais non par l'atropine.

M. C. DA SILVA E SOUZA[19], M. F. GIMENO and A. L. GIMENO[20]

Instituto de Neurobiología, Obligado 2490, Buenos Aires 28 (Argentina), 12 March 1974.

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[19] Exchange Scholarship Fellow, 'Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina' and 'Conselho Nacional de Pesquisas Brasil'. Maternidade Climerio de Oliveira, Universidade Federal da Bahía, Salvador, Bahía, Brasil.
[20] Member of the 'Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina'.

Effect of 6-Amino-Nicotinamide on the Tolerance of Mice to Hypoxic Hypoxia

In general, normal functioning of the brain and consequently the survival of the whole organism in an atmosphere low or deficient in oxygen are precluded by the high caloric requirements of the mature central nervous organ. Although anaerobic glycolysis is initially stimulated by lack of oxygen[1], the yield of energy is too low to maintain normal cellular metabolism, and anaerobic glycolysis itself will rapidly cease under these condi-

Table I. Tolerance to hypoxia (5% O_2–95% N_2) of mice treated with 6-ANA (35 mg/kg body wt.) compared to untreated controls

Treatment	Body wt. (g)[a]	Body temperature (°C)	Blood glucose (mmoles/l)	Survival time (min)
Controls Normothermic (8)[b]	31.1 ± 0.8	39.0 ± 0.3 (38.6 –39.3)	9.04 ± 0.92	3.93 ± 1.14 (2.23 –5.40)
Controls Hypothermic (8)	33.1 ± 1.0	34.9[c] ± 1.0 (32.8 –35.8)	10.41[c] ± 0.86	3.96 ± 1.58 (2.16 –7.00)
6-ANA (8)	31.1 ± 1.0	35.9[c] ± 1.4 (34.0 –38.0)	12.30[c] ± 0.96	21.70[c] ±9.29 (11.0 –30[d])

Values are means ± standard errors, with ranges in brackets. [a] Initial measurements. [b] Number of animals per group. [c] $p < 0.01$ compared to normothermic controls as determined by the one-way analysis of variance. [d] 4 out of 8 mice were still alive when the exposure to hypoxia was discontinued after 30 min.

tions. Of the glucose utilized anaerobically, a portion enters the so-called hexose monophosphate shunt in both newborn and adult animals[2–5], providing a source of hexosephosphates and triosephosphate alternative to the Embden-Meyerhof cycle. Recently 6-amino-nicotinamide (6-ANA) was found to be a specific inhibitor of the hexose monophosphate shunt[6]. In the present study, this agent was given to mice to be exposed to moderate though lethal hypoxia in an attempt to ascertain the role of this metabolic cycle during hypoxia of the brain.

In detail, male NMRI mice were given 35 mg 6-ANA/kg body weight i.p. and approximately 6 h later hypoxic survival times were determined by placing individual animals in short lengths of glass tubing through which a mixture of 5% O_2 and 95% N_2 was led. Flow was adjusted by bubbling the gas mixture through gas washing bottles. Of these tubings, 6 were mounted on a rack, and it was thus possible both to produce 'explosive' hypoxia[7] in 6 animals simultaneously and to observe them individually. The observation was discontinued when even gasping had stopped completely or when animals lived beyond 30 min of hypoxia. Body temperature was measured by inserting the tip of a thermocouple of an electronic measuring device (Tastotherm P1, Braun AG) into the rectum to a depth of about 10 mm. 6-ANA is well known to lower body temperature[8] and for appropriate hypothermic controls an open jar with 4 untreated mice each was placed in an ice bath of 0 °C temperature. The temperature inside the jar averaged 8 °C and within 30 min body temperature dropped to about 35 °C. Venous blood (10 µl) for the determination of blood glucose levels by a micro method[9] was obtained from the tail vein.

Table II. Tolerance to hypoxia (5% O_2–95% N_2) and to anoxia (100% N_2), respectively, of mice treated with 6-ANA (35 mg/kg body wt.) and made hyperglycemic in addition

Treatment	Body weight (g)	Blood glucose[a] (mmoles/l)	Survival time (min)
6-ANA + hypoxia (8)	31.3 ± 0.6	19.72 ± 2.81	22.21 ± 11.27 (8.50 — 30[b])
6-ANA + anoxia	25.9 ± 0.5	19.62 ± 2.49	0.63 ± 0.10

[a] 0.4 ml of a 10% solution of D-glucose was injected i.p. approximately 6 h after the administration of 6-ANA and blood was obtained 20 min later for the determination of blood glucose. Exposure to the experimental atmosphere was begun immediately thereafter. [b] 5 out of 8 animals were still alive at the end of the 30 min observation period.

Table I shows the results of experiments on the tolerance to hypoxia of normothermic and hypothermic controls and of mice treated with 6-ANA. The animals of both control groups rapidly succumbed to hypoxia, the majority within less than 5 min. In contrast, 6-ANA strikingly prolonged the survival periods, some animals staying alive for more than 30 min, i.e. indefinitely for practical purposes. Apparently this inhibiting agent exerts a protective action towards the effects of hypoxic hypoxia, conceivably through either one of the following ways: low body temperatures greatly depress the metabolic activity of the brain[10], thereby extending the survival periods of experimental animals exposed to hypoxia. Therefore hypothermia brought about by 6-ANA may account for its protective action. However, the data obtained with the hypothermic controls indicate that an average decrease of the body temperature from 39 °C down to about 35 °C does not yet increase the hypoxic tolerance of mice. Only in animals whose temperature was brought down to 32 °C was the hypoxic tolerance seen to rise significantly. Hyperglycemia due to 6-ANA may be another determinant in the hypoxic survival, especially since glycogen stores, at least of liver, are also raised by 6-ANA[11] and glucose levels of brain are rapidly depleted during hypoxia[12]. Hypoxic survival is, in turn, enhanced by hyperglycemia following oral intake or injections of D-glucose[7,10,13,14]. No additional effect on the hypoxic

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Table III. Effect of 6-ANA (35 mg/kg body wt.) on blood glucose levels and body wt. in fed and starved mice compared to untreated starved controls

Treatment	Body weight (g)		Blood glucose (mmoles/l)		Hypoxic survival rates[b]
	1[a]	2	1	2	
Controls, starved (8)	29.9[c] ± 0.7	27,9 ± 1.0	8.87[c] ± 0.45	7.46 ± 0.68	–
6-ANA starved (8)	30.5[c] ± 1.3	27.9 ± 1.2	9.59[c] ± 1.22	7.13 ± 0.89	6
6-ANA, fed (8)	31.1[d] ± 1.0	30.3 ± 1.1	9.50[d] ± 0.94	13.40 ± 2.41	6

[a] Measurements were made prior to (1) and 6 h after the injection of 6-ANA (2). [b] Number of animals alive after 30 min of hypoxic hypoxia; the untreated controls were not subjected to hypoxia. [c] The values of body weight and blood glucose of untreated starved controls and starved treated animals were submitted to a two-way analysis of variance. There was a significant effect of time on weight and blood glucose in both groups ($p < 0.01$), differences between groups were not significant, however. [d] Differences were significant ($p < 0.01$) according to Students t-test for paired observations.

tolerance of 6-ANA-treated mice was seen with an injection of D-glucose, even though blood glucose levels were more than twice as high, compared with controls (Table II). Likewise the combined effects of 6-ANA and hyperglycemia failed to enhance the tolerance to comlpete anoxia.

Finally another attempt was made to ascertain the implications of blood glucose levels in the hypoxic tolerance of 6 ANA-treated mice (Table III). When mice given the usual dose of 6-ANA were starved for 6 h, blood glucose levels were similarly reduced to those of starved untreated controls. Thus 6-ANA-induced hyperglycemia, which is primarily brought about by concomitant rises in serum corticosterone and adrenaline[11], may be partly due to an impaired alimentary glucose tolerance. Even more important, however, is the identical hypoxic tolerance in both 6-ANA-treated groups.

Little or no effect on the hypoxic survival times was anticipated to arise from the blocking of the hexose monophosphate shunt in view of its minor contribution to the overall metabolism of glucose[2–5]. Indeed, rather than impairing the hypoxic tolerance, 6-ANA was found to effect the opposite, indicating that even hypoxia does not challenge this alternative glycolytic pathway. Furthermore, the results demonstrate that the enhanced hypoxic tolerance is not related to systemic effects of 6-ANA like hyperglycemia or hypothermia. Although an overall depression of cerebral metabolic activity similar to the one observed with anaesthetics[15] cannot be ruled by the present study, the data may also be consistent with the assumption that anaerobic glycolytic flux is increased by 6-ANA. During brain ischemia, lactate levels of 6-ANA-treated animals rise more rapidly and to higher levels than in control animals[16], possibly as a result of enhanced glycolysis. Whether such a mechanism actually applies during hypoxia remains open at present. That anaerobic utilization of glucose by brain may be effectively stimulated by pharmacological agents, is nevertheless an intriguing possibility, deserving close attention, also in view of possible implications in human medicine[17].

Zusammenfassung. Eine einmalige Injektion von 6-Aminonicotinamid (6-ANA) steigerte die Sauerstoffmangelresistenz von Mäusen in einer Atmosphäre mit 5% O_2 und 95% N_2. Hyperglykämie und Hypothermie, systemische Wirkungen von 6-ANA, konnten als Ursachen der gesteigerten Resistenz ausgeschlossen werden. Es wird deswegen vermutet, dass 6-ANA die anaerobe Utilisation von Glucose im Gehirn fördert.

H. H. Berlet

Institute of Pathochemistry and General Neurochemistry, University of Heidelberg, Postfach 104340, D-69 Heidelberg (Federal Republic of Germany),
29 March 1974.

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The Effect of Salicylates on Plasma Fibrinolytic Activity in the Rat

Circulating antiplasmins have been postulated to be in a dynamic equilibrium with plasmin (fibrinolysin) and to compete with fibrin for its active site[1]. Sodium salicylate was reported to inhibit the breakdown of fibrin by plasmin in vitro[2,3]. On the contrary several synthetic antiinflammatory agents have been shown to induce fibrinolysis in vitro[4,5]. In this paper the effect of administering sodium salicylate i.p. or acetylsalicylic acid orally on the plasma fibrinolytic activity of rats is examined.

Methods. Male Wistar rats weighing 180–250 g were injected i.p. with sodium salicylate dissolved in phosphate buffer 0.12 *M*, pH 7.4 at doses of 50 mg, 100 mg and 150 mg/kg. Acetylsalicylic acid was given orally in the same doses after mixing with tragacanth powder in the ratio 2:1 to make a gummy suspension. Control rats were given 0.9% sodium chloride solution i.p. or orally. After

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